REMARKS

Claims 91, 99 and 100 have been amended to more distinctly claim the subject matter of the invention. Applicants reserve the right to file continuation and/or divisional applications containing claims encompassing the canceled subject matter. As will be discussed in further detail below, the amended claims contain no new matter and are supported by the specification.

I. The Rejections Under 35 USC §102

Claims 91-94 and 97-98 are rejected under 35 U.S.C. §102(b) as being anticipated by Scheele (US Patent 5162209 November 10, 1992). Applicants disagree. It is well established case law that

> [U]nless a prior art reference discloses within the four corners of the document not only all of the limitations claimed but also all of the limitations arranged or combined in the same way as recited in the claim, it cannot be said to prove prior invention of the thing claimed and, thus, cannot anticipate under patent statute. *Net MoneyIn Inc. v. VeriSign Inc.*, 545 F.3d 1359, 88 U.S.P.Q.2d 1751

As will be set forth below, this is clearly not the case.

The Office Action specifically states on pages 6-8:

> (A) The reply asserts that the rejection does not provide that the primers be chemically modified (p. 12 last paragraph). The reply asserts that the teaching of labeling primers with radioactive labels would not lead one of skill in the art to consider the primers to be modified primers (p. 12 last paragraph). The reply asserts that there is a phosphorus moiety at each site regardless of whether it is a normal or radioactive version of P and thereby the labeled primer has the same chemical properties as an unlabeled primer (p. 12 last paragraph). The reply asserts that further, the Scheele's primer is not labeled but rather that the element is a radioactively labeled template used for binding and extension (p. 12 last paragraph). The reply asserts that after the extension reaction digestion of the unlabeled primer by RNAse H renders the Poly(dC) in ss form that is a substrate for exonucleolytic digestion by T4 (p 12 last paragraph). The reply asserts that therefore it is the removal of the radioactively labeled target and not the primer (p. 13 1st paragraph).

> These arguments have been fully reviewed but have not been found persuasive.
>
> The claims are drawn to "chemically-modified primers", however, the claims do not require that these primers to have the same or different chemical properties. Rather, these primers have a chemical attachment, which is different from a nucleic acid without a chemical attachment. This attachment would be the radioactive label. The reply asserts that it is the target, which is labeled not the primer. However, Scheele et al. teaches that the primer includes a portion of nucleotides that are complementary to an oligonucleotide tail (Column 3 lines 25-35). Scheele et al. teaches that this tail comprising nucleoside triphosphates (Column 3 table 1 and column 4 lines 5-10). The method does not require that the chemically modified primer to be removed, but rather requires that the sample be contacted with a primer that has been chemically modified. This term is being broadly interpreted as requiring that the primer being present with a radioactive labeled nucleotide. Herein Scheele et al. teaches that the primer is mixed with a radioactive nucleotide (Column 8 lines 5-40).

Applicants disagree. In the first place, as previously noted, claim 91 requires the use “chemically modified primers”, an element that is missing from the process of Scheele. The presence of radioactively labeled nucleotides in a primer would not be lead individuals skilled in the art to consider the primers to be “modified’ primers. Other than being a different isotope, there is a Phosphorus moiety at each site, whether it is a normal or radioactive version of P, thereby giving the labeled primer the same chemical properties as an unlabeled primer. Secondly, a close reading of the section of Scheele cited in the previous response as support for “chemically modified primers” reveals that it is with regard to a riboG tail hybridized to a poly(dC) tail as depicted in Figure 5. It can be seen that the primer is not labeled (or modified) in Scheele, but rather, a radioactively labeled template is used for binding and extension. Thus there is no description of a chemically modified primer in Scheele and it does not anticipate claim 91 which requires such a modification.

> (B) The reply asserts that all of the steps are taking place under isostatic conditions and therefore the binding, extension, removal and binding of a new primer on a regenerated primer binding site are taking place simultaneously (p. 13 1st full paragraph).

> This has been fully reviewed but has not been found persuasive.
>
> The claims do not require that the steps be done simultaneously. Further the claims require that the steps be done under isostatic conditions of temperature, buffer, and ionic strength. However, the claims do not require that the isostatic conditions be identical in each step. Therefore Scheele et al. teaches performing steps a-d in solutions with particular temperature and buffers and as such teaches the requirements for the claim.

Applicants respectfully traverse the rejection. It is Applicants' position that since the claim already included the limitation "under isostatic conditions of temperature, buffer and ionic strength" in claim 91 that it would be evident that it would by definition be uniformly isostatic. However, in order to more distinctly claim the invention, steps (c) and (d) of claim 91 have been amended to more clearly emphasize that step (d) takes place as a consequence of allowing said mixture to react under isostatic conditions. Applicants wish to specifically point out that the phrase "and thereby producing" has been amended to recite "to produce".

The Office Action on pages 7 and 8 states:

> (C) The reply asserts that although Scheele uses an RNAse H step to remove primer sequences, the nucleotide acid sequence thereby rendered single stranded are not used for new primer binding events (p. 13 1st full paragraph). The reply asserts that Scheele teaches away from this method because he teaches the simultaneous presence of a nuclease that will digest away the sequences that could otherwise provide a primer binding site (p. 13 1st full paragraph). The reply asserts that the RNase H digestion step does not provide means for amplification but is only used to render the ds nucleic acid into a blunt ended form (p. 13 1st full paragraph). The reply asserts that the thermal denaturation step of the PCR reaction of Scheele does not remove a portion of the primer but instead removes the entire extended nucleic acid (p. 13 1st full paragraph).
>
> This argument has been fully reviewed but has not been found persuasive.
>
> The reply seems to be asserting that the same primer binding event must occur, however, the claim has not been limited to such a step. Specifically step d requires digestion with RNase H wherein the removal allows for another DNA

> molecule to be produced. Herein in the instant case, Scheele et al. teaches the amplification via PCR. Scheele et al. teaches a sample of ds cDNA is prepared and added to its RNA primer with its DNA tail extension intact and excess RNA primers and excess oligo(dT). Taq and dNTPs are further added. The mixture is then subjected to PCR and the RNAse is added. As such Scheele et al. teaches that multiple copies of the DNA molecule of interest are produced. The applicant seems to be asserting that the main difference between Scheele et al. and the claimed method is that the claim method requires the addition of RNase H before multiple copies are produced. However, the claim has a larger breadth than this limitation. Step d only requires the digestion of the substrate with RNase H so that the substrate is capable of anther primer binding event to occur. The steps recited do not limit the last step to a positive recitation of removing the RNA segment with RNase H and then producing another DNA molecule by performing steps a-d.

In response, Applicants respectfully point out that reference is made in the Office Action to Scheele concerning the use of RNase to digest RNA moieties in a primer and the use of PCR to make multiple copies. However, even though Scheele describes the use of RNase H for digestion and the use of PCR would involve another priming event take place, there is no description in Scheele of a priming event being due to the removal of a primer portion of the complementary copy produced as recited in claim 91. There is no description in Scheele itself that removal of the primer portion (e.g. by RNase H digestion) "render[s] said primer binding site available for a new priming event to take place" as recited in claim 91. Since RNase H does not perform this function in the method of Scheele, they are forced to substitute another method, thermocycling, for regeneration of a primer binding site.

Applicants still assert that there is a teaching away from the use of removal of primer sequence (e.g., by RNase H digestion) to generate a new priming site since as seen in Figure 5, the next step after removal of the $r(G)_n$ primer segment by RNase H, is a further removal of the homopolymeric $d(C)_n$ segment by T4 DNA polymerase; quite clearly this step will not allow the $d(C)_n$ segment to be used again for a priming event. It isn't a question that the removal of sequence in claim 91 is allowing the "same primer binding event to occur", but rather that RNase H digestion in Scheele is not responsible for allowing any particular primer binding events to take place and only denaturation is

used to allow further priming events. No connection between RNA removal and rendering the primer binding site available for another priming event is present in Scheele. These are independent events where a) RNase H is used to generate a single-stranded "tail' that is subsequently removed by T4 DNA polymerase and b) PCR carries out a series of subsequent primer binding events but neither a) or b) is dependent on the other. If a primer is then used in PCR reaction in the remaining double-stranded portion, this would take place in the double-stranded portion that would be totally independent of whether there was removal of the homopolymeric segments by RNase H +T4 DNA polymerase or not.

Applicants assert that claim 91 is clearly distinguished from the Scheele reference. However, in order to further prosecution and to emphasize features of the present method that is distinct from those of Scheele, claim 91 has been amended to emphasize the connection between removal of primer sequence and subsequent primer binding events that results in production of more copies.

Applicants wish to point out that claim 91, as amended, requires that (a) another priming binding event takes place because removal of the primer portion from the complementary copy produced in step (c) takes place. Further, as written, only "one or more specific chemically-modified primers each of which primer is substantially complementary to a distinct sequence of said specific nucleic acid" are the only sequences present in the mixture provided in step (b) of claim 91 and consequently they would be the only primers available for "a new primer binding event". This gives a particular reason for the removal of these sequences since their removal would then allow the regeneration of the same primer binding site that was used in step (c) and thereby a new priming event should automatically undergo the same extension and subsequent removal described for the first primer binding event since the mixture provided in the contacting step (b) contains the nucleic acid producing catalyst.

Claims 92-94 and 97-98 depend from claim 91. Therefore, arguments made with respect to 91 apply to these claims as well.

In view of the above arguments and the amendment of claim 91, Applicants assert that the rejection of claims 91-94 and 97-98 under 35 USC §102(b) have been overcome. Therefore, Applicants respectfully request that these rejections be

withdrawn.

II. The Rejections Under 35 USC §103

Two rejections under 35 USC §103 were made. The rejections and Applicants response are set forth below.

A. The Rejection of Claims 95-96

Claims 95-96 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Scheele (US Patent 5162209 November 10, 1992) in view of Gelfand et al. (US Patent 5374553 December 20,1994). The Office Action specifically states:

> Therefore it would be prima facie obvious to modify the in vitro translation method of Scheele et al. to have a phosphorothioate (sulfur heteroatoms) on the 3' end of the primers as taught by Gelfand et al. in order to maintain the primers during the PCR step of Scheele et al (Column 8 lines 58-60) to produce multiple copies of the nucleic acid of interest. The ordinary artisan would be motivated to modify the primer of Scheele et al. to include the phosphorothioate (sulfur heteroatoms) of Gelfand et al., because Gelfand et al. teaches that the addition of phosphorothioate to the primers ends allows the primers to be more resistant to degradation (Column 13 lines 15-20). Therefore the ordinary artisan would be motivated to modify the primers of Scheele et al. to include the phosphorothioate (sulfur heteroatom) of Gelfand et al. because primers resistant to degradation can be maintained longer in a PCR and therefore more copies of the original nucleic acid may be produced.

Applicants respectfully traverse the rejection. It is Applicants' position that even if there was motivation to combine the two cited references, the claimed method would not result. As previously noted, there are three important distinctions between the Scheele method and the method of the present invention: (a) the primers used in the method of the present invention are not chemically modified; (b) the method of the present invention is conducted in its entirety under isostatic conditions of temperature, buffer and ionic strength and (c) removal of the primer portion of the complementary sequence in the method of the present invention is connected to allowing the template to be used for a new priming event. Gelfand would not fill in the gaps. As noted in the previous response, Gelfand repeats some of the same deficient practices of Scheele in

that this reference is also concerned with PCR thermal cycling rather than an isostatic mode of amplification.

In view of the above arguments, Applicants assert that the rejection of claims 95-96 under 35 USC §103 have been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

B. The Rejection of Claims 99-103

Claims 99-103 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Scheele (US Patent 5162209 November 10, 1992) in view of Reischl et al. (US Patent 5474916 December 12,1995). The Office Action specifically states:

> The reply asserts that although Scheele teaches that digestion by RNAse H of an RNA primer can render a template single stranded, Scheele does not teach the use of the single stranded portion created by this process as being used as a primer binding site (p. 18 last paragraph).
>
> These arguments have been fully reviewed but have not been found persuasive.
>
> As stated above, the claims are not limited to the production of the second copy of the nucleic acid by the steps of a-d. Rather the claims are limited to the production of multiple copies of the nucleic acid and the removal of loop structures.
>
> The reply asserts that Scheele et al. does not teach a loop (p. 18 last paragraph-19 1st paragraph). The reply asserts that the loop of Reischl et al. would not be combined as it is not certain how RNAse H would remove the loop portion because RNAse H has no affinity for ss DNA (p. 19 2nd paragraph). The reply asserts that the loop of Resichel is preexisting and is not formed upon hybridization (p. 19 2nd paragraph). The reply asserts that there is no explanation as to why the digestion of the tail that contains a non-complementary loop would promulgate another primer binding event (p. 19 2nd paragraph).
>
> These arguments have been fully reviewed but have not been found persuasive.
>
> The claim does not require RNAse H. Further the RNAse H is not digesting the loop portion but rather it would digest the tail end of which the loop is at the end. Once this portion is digested the loop would be removed from the complex. The reply asserts that the loop must be formed at the point of

> hybridization. However, the loop of Reischl et al. is formed at the point of hybridization. Reischl et al. teaches the P1 nucleotide promotion (e.g. the loop structure) has a single stranded region that is either not at all or only to a limited extent complementary to the template (Column 4 lines 1-30). As such this region that has a loop upon hybridization to the template is not completely complementary to the template so the region that is not complementary forms the loop structure.

Applicants respectfully traverse the rejection. First, Applicants disagree with the assertion that the claims are just limited to the production of multiple copies of the nucleic acid and the removal of loop structures. However, in order to advance prosecution and to emphasize features of the present method that is distinct from those of Scheele, step (d) and the "thereby" portion of claim 91 have been amended to emphasize the connection between removal of the loop structure or structures and subsequent primer binding events that results in production of more than one copy of the specific nucleic acid of interest. Further, it is conceded in the Office Action that Scheele does not teach a method where the primers form a loop structure.

Although Reischl does teach loop structures, this reference would not in Applicants view cure the other deficiencies of Scheele noted above: lack of isostatic conditions and lack of connection between removal of loop structure and subsequent primer binding events. Reischl merely discloses a promoter based amplification system which actually contains two primers. There is certainly no suggestion or disclosure of an in vitro transcription system under isostatic conditions of temperature, buffer and ionic strength. At best, the combination of Reischl and Scheele would result in an in vitro PCR based transcription method using at least one primer that forms on or more loop structures.

Claims 100-103 depend from claim 99. Therefore, arguments made with respect to claim 99 would apply to these claims as well.

In view of the amendment of claim 99 and the above arguments, Applicants assert that the rejection of claims 99-103 under 35 USC §103 has been overcome. Therefore, Applicants respectfully request that this rejection be withdrawn.

III. Conclusion

In view of the foregoing, Applicants assert that the claims are now in condition for allowance. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned at (914) 712-0093 if she has any questions.

Respectfully submitted,

Date: _____

Cheryl H. Agris, Reg. No. 34,086
Customer No. 25538
PO Box 8495
Pelham, NY 10803
Tel: (914) 712-0093
Fax: (914) 219-1063

Cheryl Agris 7/31/03 3:29 PM
Formatted: Font:Not Bold, Not Italic